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(54) Title: THE USE OF NONIMMUNOSUPPRESSIVE [$G(G)-HYDROXY-N-METHYL-L-LEUCINE[4]] CYCLOSPORIN DERIVATIVES FOR TREATING HAIR LOSS

A-Abu-B-HMeLeu-C-D-E-F-G-H-I (1)

(57) Abstract: The present invention discloses a hair growth promoting agent comprising a cyclosporin derivative having an excellent hair growth stimulating ability with little immunosuppressive effect as an active ingredient. In accordance with the invention, there is provided a hair growth promoting agent comprising a cyclosporin derivative represented by Chemical Formula 1 below. The derivatives of the invention were prepared by derivatization of cyclosporins at the amino acid residue 4, N-methyl-L-leucine, and hair growth promoting effects thereof were examined. Such a hair growth promoting agent, comprising a cyclosporin derivative as an active ingredient, exhibits an excellent hair growth effect, while it shows very weak immunosuppressive activity, compared to unmodified cyclosporin A.

THE USE OF NONIMMUNOSUPPRESSIVE [γ -HYDROXY-N-METHYL-L-LEUCINE^4] CYCLOSPORIN DERIVATIVES FOR TREATING HAIR LOSS

Technical Field

The present invention relates to a hair growth promoting agent comprising a [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivative, having an excellent hair restoring effect with non-immunosuppressive activity, as an active ingredient.

Background Art

On average, the human scalp contains about 100,000 to 150,000 hairs. Each hair has three main stages of growth: anagen, catagen and telogen, after which the hair falls out. This hair growth cycle is repetitive and the duration of one cycle is different from other cycles, ranging from approximately 3 to 6 years. Thus, the average adult normally loses about 50 to 100 hairs every day. In general, alopecia refers to a phenomenon wherein duration of the anagen growth phase is shortened and the percentage of hairs in the catagen and telogen phases increases, whereby the number of lost hairs is abnormally increased.

There are many theories to explain loss of hair, including for example, poor blood circulation, excessive functioning of male sex hormone, excessive production and secretion of sebum, deterioration of scalp by peroxides, bacteria, etc., hereditary factors, aging, stress, etc. However, explicit mechanisms have not been revealed. Recently, the population suffering from hair loss is tending to increase, since changing dietary habits and stress imposed on individuals due to modern social environments, etc. have increased. Also, the age of the individuals affected by alopecia is dropping and furthermore, the population of female alopecia sufferers is rising.

One of preparations which are most commonly used for treatment and prevention of alopecia is one that contains minoxidil. There are two hair-regrowth agents which have received approval from the U.S. Food and Drug Administration, and minoxidil is one of those approved hair-regrowth agents. Minoxidil was originally developed as a hypertension drug for the purpose of reducing blood pressure. However, when using this drug, as a side effect, a trichogenous effect was observed and thereafter, this drug became famous as a hair-regrowth agent.

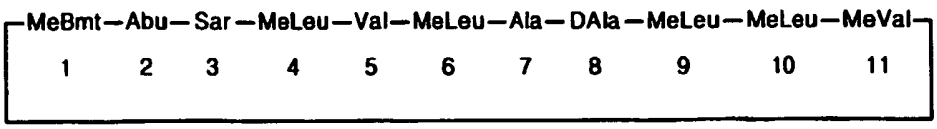
Although mechanisms by which minoxidil works as a hair-regrowth agent is not clearly understood, it is inferred that minoxidil increases blood flow by expansion of blood vessels, whereby roots of hairs are supplied with more nutrition and eventually, growth of hairs are promoted.

Such a model of blood flow increase has been indirectly supported by a recent report that minoxidil enhances the expression of vascular endothelial growth factor (VEGF), a growth factor associated with vasodilatation in the dermal papilla which is a main cell making up the hair roots (Br. J. of Dermatol., 1998, 138:407-411). Also, other than the vasodilative effect of the minoxidil in the hair-restoring mechanism, it has been reported that minoxidil promotes activation of dermal papilla cells in the roots of hair incubated *in vitro*, and growth of hair follicles in a tissue culture of follicles *in vitro* (Skin Pharmacol., 1996, 9:3-8 and J. Invest. Dermatol., 1989, 92:315-320). These facts indicate that minoxidil may work directly on the roots of hair as a growth factor.

In addition, finasteride, a main component of Propecia which has started to be sold by Merck, is used for treatment of alopecia. It inhibits conversion of the male hormone testosterone into dihydrotestosterone, which is a more potent male hormone than testosterone. On December of 1997, the 1 mg finasteride tablet was approved by the US FDA as a hair-regrowth agent for treatment of male pattern hair loss in men only, and is now commercially available. In clinical studies, it has been demonstrated to have a significant trichogenous effect. However, there has been a report that finasteride may inhibit male sexual function as a side effect (J. Am. Acad Dermatol., 1998, 39:578-589). Since neither finasteride nor minoxidil show superior effect in clinical tests, and there is concern about side effects, many researches are conducted to develop a new and improved hair-regrowth agents.

The cyclosporin family of drugs has immunosuppressive activity. It is also effective to inhibit growth of virus, fungus, protozoan, etc. and has various physiological effects such as nephrotoxicity, hepatotoxicity, hypertension, enlargement of periodontium, trichogenous effect, and so on, as side effects (Advances in Pharmacol., 1996, 35:114-246 and Drug Safety, 1994, 10:310-317). Cyclosporin A, a representative cyclosporin, is a cyclic peptide having the following Chemical Formula, which comprises 11 amino acids, including several N-methyl amino acids and D-alanine at No. 8 residue.

[Structure Formula 1]



where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine, Abu is L-α-aminobutyric acid, Sar is sarcosine, MeLeu is N-methyl-L-leucine,Val is L-valine, Ala is L-alanine, DAla is D-alanine, and MeVal is N-methyl-L-valine.

The amino acid form of cyclosporin A of the above Chemical Formula 1 is L-configuration, unless otherwise specified. The residue numbering of amino acids starts from MeBmt and proceeds clockwise, i.e. 1 for MeBmt and 11 for the last MeVal (N-methyl-L-valine) as shown in the Structure Formula 1. Nomenclature of various derivatives including cyclosporins A to Z, follows methods commonly used (Helv. Chim. Acta, 1987, 70:13-36). For example, if Abu, the residue No. 2 of cyclosporin A is substituted with L-alanine, L-threonine, L-valine or L-norvaline, the derivatives thus prepared are named cyclosporin B, cyclosporin C, cyclosporin D or cyclosporin G, respectively. Further, when the amino acid residues of the cyclosporin derivatives differ from those of cyclosporin A, the derivatives are named by describing the substituent. For example, if cyclosporin A is modified, the derivative is named [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A, and if cyclosporin B and cyclosporin C are modified, the derivatives are named [γ-hydroxy-N-methyl-L-leucine^4] B and [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin C, respectively. If sarcosine, being the residue No. 3 of cyclosporin A, is substituted with N-methyl-D-Abu^3 or N-methyl-D-Nva^3, the derivatives thus prepared are named [N-methyl-D-Abu^3] cyclosporin A or [N-methyl-D-Nva^3] cyclosporin A, respectively. Also, in the case that two or more residues are substituted, the derivatives are named in a similar manner. For example, if both of residues No. 3 and No. 4 are substituted, the derivative is named [N-methyl-D-alanine^3] [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A. If both of residues No. 4 and No. 9 are substituted, the derivative is named [γ-hydroxy-N-methyl-L-leucine^4] [γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A. Also, if substitution at the residues No. 4 and No. 7 concurs, the derivative is described as [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, [$^7\psi^8$ CS-NH] cyclosporin A.

Meanwhile, a peptolide is produced by conversion of an amide bond to an ester bond in a cyclosporin molecule due to substitution of, for example, the No. 8 residue, D-alanine, with hydroxy acid. The hydroxyl group has a general formula,

-O-RCH-CO-, in which R is a C_{1-4} alkyl group. The most preferred hydroxyl group is hydroxyisovaleric acid (hereinafter abbreviated to Hiv). Representative peptolides include [L-threonine^2][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A, [L-Thr^2][D-Hiv^8][Leu^{10}] cyclosporin A, [L-Thr^2][Ile^5][D-Hiv^8][Leu^{10}] cyclosporin A, [L-Thr^2][Leu^4][Leu^5][D-Hiv^8][Leu^{10}] cyclosporin A, and [L-Thr^2][Sar^3][Leu^5][D-Hiv^8][Leu^{10}] cyclosporin A, capable of being obtained by employing Cylindrotrichum Bonorden NRRL 18230.

Regarding the peptolide in which the residue No. 8, D-alanine is substituted with D-hydroxyisovaleric acid, forming an ester bond in its amino sequence, if the residue No. 4, N-methyl-L-leucine is converted to [γ-hydroxy-N-methyl-L-leucine^4], the cyclosporin derivative is described as [L-threonine^2] [L-leucine^5] [γ-hydroxy-N-methyl-L-leucine^4] [D-hydroxyisovaleric acid^8] [L-leucine^{10}] cyclosporin. Further, as for a derivative of cyclosporin which is substituted with sulfur instead of a carbonyl oxygen at the amino acid residue 7, the name of the derivative may be cyclosporin 7-thioamide or [$^7\psi^8$ CS-NH] cyclosporin, according to different references (Helv. Chim. Acta. 74: 1953-1990, 1991; J. Org. Chem. 58: 673-677, 1993; J. Org. Chem. 59: 7249-7258, 1994). In addition, a common method for abbreviating amino acids is employed, that is, N-methyl-L-leucine is abbreviated by MeLeu, N-methyl-L-isoleucine by MeIle, N-methyl-L-Valine by MeVal, N-methyl-L-alanine by MeAla, N-methyl-L-norvaline by MeNva, L-leucine by Leu, L-isoleucine by Ile, sarcosine by Sar, L-serine by Ser, L-valine, Val, L-alanine by Ala, D-alanine by DAla, L- aminobutyric acid by Abu, L-threonine by Thr, and L-norvaline by Nva. In the invention, the term 'cyclosporin deriatives' generally refers to [γ-hydroxy-N-methyl-L-leucine^4] cylosporin derivatives which are substituted at residue No. 4 of cyclosporin with γ-hydroxy-N-methyl-L-leucine^4, having an excellent hair restoring effect with non-immunosuppressive activity.

So far, possible development of cyclosporin as a hair-regrowth agent has been studied by many research groups. Particularly, researches involving animal hair regrowth tests (Arch, Dermatol. Res., 1996, 288:408-410), human alopecia areata (J. Am. Acad. Dermatol., 1990, 22:242-250), human male pattern alopecia (J. Am. Acad. Dermatol., 1990, 22:251-253 and Skin Pharmacol., 1994, 7:101-104), and inhibition effect of hair loss by chemotherapy in animal models (Clin. Lab. Invest., 1995, 190:192-196 and Am. J. Pathol., 1997, 150:1433-1441) have been widely conducted. In comparative experiments on mouse's back, it is shown that cyclosporin has a hair regrowth effect about 100 times superior to minoxidil.

Based on such findings, there have been attempts to utilize cyclosporin as a treatment for male pattern alopecia, and many applications for patents have been filed.

For example, Japanese Patent Publication Kokai Nos. Sho 60-243008, Sho 62-19512 and Sho 62-19513 disclose use of cyclosporin derivatives as a hair regrowth agent. Also, European Patent Publication No. 0414632 B1 discloses cyclosporin derivatives modified at residue No. 8, and PCT Patent Publication No. WO 93/17039 discloses isocyclosporin provided as a hair regrowth agent. In U.S. Patent No. 5,807,820 and U.K. Patent No. 2,218,334 A, preparations containing cyclosporin with excellent transdermal absorption are suggested for new application of a hair regrowth agent.

Disclosure of the Invention

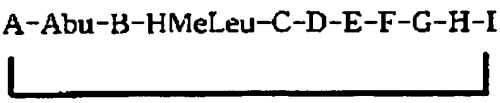
Therefore, the present invention has been made in view of the above problems associated with side effects of cyclosporin A, and based on the knowledge that the hair restoring effect of cyclosporin is not always in line with its immunosuppressive activity (Iwabuchi et al., Dermatol. Sci., 1995, 9:64-69), and it is an object of the present invention to provide a novel hair growth promoting agent prepared by a variety of molecular modification from cyclosporin, which retains a hair restoring effect, while its immunosuppressive activity is lost.

Approaches similar to the above have been actively made to develop agents for the treatment of acquired immunodeficiency syndrome (AIDS) caused by infection with HIV virus. Those agents are cyclosporin derivatives retaining their inhibition activity against HIV, while having reduced immunosuppressive activity. Esecially, [γ-hydroxy-MeLeu^4] cyclosporin A, [MeIle^4] cyclosporin A and [MeVal^4] cyclosporin A are cyclosporin derivatives with modifications at amino acid residue No. 4, which are disclosed in references as novel anti-HIV agents (U.K. Patent No. 484,281 A2, U.S. Patent No. 5,767,069, J. Virol., 1995, 69:2451-2461, and J. Antibiotics, 1996, 49:781-787). The inventors examined hair restoring effects and immunosuppressive activities with respect to a variety of cyclosporin derivatives including the derivatives substituted at residue No. 4 with γ-hydroxy methylleucine, methylisoleucine, methylvaline, leucine, or isoleucine, instead of methylleucine, the original residue No. 4, with γ-hydroxy methylleucine being the residue similar in structure to the original MeLeu. Among the above

derivatives tested, [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A was found to be a novel hair growth promoting agent which uniquely has a hair restoring effect with no immunosuppressive activity. This finding led to searches for derivatives other than cyclosporin A. As one of the results, it was found that [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A also has a hair restoring effect while the immunosuppressive activity is reduced. Accordingly, the invention is directed to [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivatives.

The present invention is directed to a hair growth promoting agent comprising a [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivative represented by Chemical Formula 1 below, having an excellent hair restoring effect with non-immunosuppressive activity, as an active ingredient.

[Chemical Formula 1]



wherein:

A represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine (MeBmt), (2S,3R,4R,6E)-3-sulfhydryl-4-methyl-2-(methylamino)-6-octenoic acid or (2S,4R,6E)-3-oxo-4-methyl-2-(methylamino)-6-octenoic acid;

Abu represents L-α-aminobutyric acid (Abu);

B represents a D-amino acid represented by the general formula 1,

[General formula 1]

CH_3NH-CH(R)-COOH

in which,

R is one selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino, and X-R' represented by the general formula 2 below,

[General formula 2]

-X-R'

in which,

X is oxygen or sulfur, and

R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one

or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

C represents L-valine or L-norvaline;

D represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine;

E represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

F represents D-2-hydroxyisovaleric acid or a D-amino acid represented by the general formula 3,

[General formula 3]

-NH-CH(CH_2R)-COOH

in which,

R is hydrogen or X-R' represented by the general formula 4,

[General formula 4]

-X-R'

in which,

X is oxygen or sulfur, and

R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

G represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine;

H represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

I represents N-methyl-L-valine or L-valine.

The preferred derivative of the above Chemical Formula 1, having an excellent hair restoring effect with non-immunosuppressive activity, is a [γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin derivative represented by Chemical Formula 2.

[Chemical Formula 2]

MeBmt-Abu-A'-HMeLeu-Val-MeLeu-B'-C'-D'-MeLeu-MeVal

wherein:

MeBmt represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine;

Abu represents L-α aminobutyric acid (Abu);

A' represents N-methyl-D-alanine, D-2-(methylamino)pent-4-enoyl, N-methyl-D-aminobutyric acid, N-methyl-D-norvaline, D-2-(methylamino)hexa-4-ynoyl, D-2-(methylamino)pent-4-ynoyl, D-2-methylthio-sarcosine, N-methyl-O-propenyl-D-serine or N-methyl-D-serine;

HMeLeu represents γ-hydroxy-N-methyl-L-leucine;

Val represents L-valine;

MeLeu represents N-methyl-L-leucine;

B' represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

C' represents D-2-hydroxyisovaleric acid, or a D-amino acid represented by the general formula 3,

[General formula 3]

-NH-CH(CH$_2$R)-COOH

in which,

R is hydrogen or X-R' represented by the general formula 4,

[General formula 4]

-X- R'

in which,

X is oxygen or sulfur, and

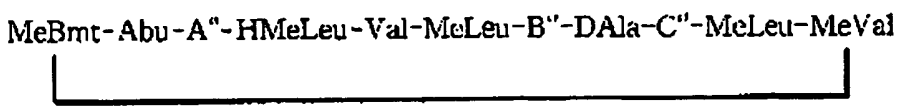
R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

D' represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

MeVal represents N-methyl-L-valine.

The more preferred derivative of the above Chemical Formula 1, having an excellent hair restoring effect with non-immunosuppressive activity, is a [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivative represented by Chemical Formula 3.

[Chemical Formula 3]



wherein:

MeBmt represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine;

Abu represents L-α aminobutyric acid (Abu);

A" represents N-methyl-D-alanine, D-2-(methylamino)pent-4-enoyl, N-methyl-D-aminobutyric acid, N-methyl-D-norvaline, D-2-(methylamino)hexa-4-ynoyl, D-2-(methylamino)pent-4-ynoyl, D-2-methylthio-sarcosine, N-methyl-O-propenyl-D-serine or N-methyl-D-serine;

HMeLeu represents γ-hydroxy-N-methyl-L-leucine;

Val represents L-valine;

MeLeu represents N-methyl-L-leucine;

B" represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

DAla represents D-alanine;

C" represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

MeVal represents N-methyl-L-valine.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [D-2-(methylamino)pent-4-enoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [N-methyl-D-Abu^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [N-methyl-D-Norvaline^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [D-2-(methylamino)hexa-4-ynoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [D-2-(methylamino)pent-4-ynoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [D-2-methylamino-Sar^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [N-methyl-O-propenyl-D-serine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [N-methyl-D-serine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [γ-hydroxy-N-methyl-L-leucine^4][alanine thioamide^7, $^7\psi^8$ CS-NH] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [γ-hydroxy-N-methyl-L-leucine^4][D-serine^8] cyclosporin A as an active ingredient.

In accordance with another aspect of the present invention, there is provided a hair growth promoting agent comprising [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A as an active ingredient.

In accordance with yet another aspect of the present invention, there is provided a hair growth promoting agent whose composition comprising a [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivative may be formulated in the form of liquid formulations, sprays, gels, pastes, emulsions, creams, conditioners or shampoos.

Brief Description of the Drawings

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description

taken in conjunction with the accompanying drawing, in which:

Fig. 1 is a HPLC chromatogram of [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A (having a retention time of 18 to 20 min);

Fig. 2 is a ^{1}H-NMR spectrum of [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A;

Fig. 3 is a ^{13}C-NMR spectrum of [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A;

Fig. 4 is a HPLC chromatogram of [γ-hydroxy-N-methyl-L-leucine^4] [alanine thioamide^7, $^7\psi^8$ CS-NH] cyclosporin A (having a retention time of around 16. 2 min);

Fig. 5 is a ^{1}H-NMR spectrum of [γ-hydroxy-N-methyl-L-leucine^4] [alanine thioamide^7, $^7\psi^8$ CS-NH] cyclosporin A;

Fig. 6 is a ^{13}C-NMR spectrum of [γ-hydroxy-N-methyl-L-leucine^4] [alanine thioamide^7, $^7\psi^8$ CS-NH] cyclosporin A;

Fig. 7 is a ^{1}H-NMR spectrum of [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A;

Fig. 8 is a ^{13}C-NMR spectrum of [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A;

Fig. 7 is a ^{1}H-NMR spectrum of [D-2-(methylamino)pent-4-ynoyl^3] cyclosporin A;

Fig. 8 is a ^{13}C-NMR spectrum of [D-2-(methylamino)pent-4-ynoyl^3] cyclosporin A; and

Fig. 9 is a FAB-MS spectrum of [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A with a peak at m/z 1286.0 representing [M (peptolide derivative)+Na].

Best Mode for Carrying Out the Invention

Hereinafter, the present invention will be described in detail, in conjunction with various examples. These examples are provided only for illustrative purposes, and the present invention is not to be construed as being limited to those examples.

With the aim of developing a novel hair growth promoting agent having a hair restoring effect without immunosuppressive activity, the present inventors prepared a variety of cyclosporin derivatives via chemical synthesis and

modification, and hair growth promoting effects thereof were examined. As a result, most of cyclosporin derivatives showed a considerable decrease in their hair restoring effects, compared to unmodified cyclosporin. However, [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivatives, which are prepared by modifying cyclosporin using microorganisms, retain their hair restoring effects, while having no immunosuppressive activity.

Example 1

Preparation of [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A

Step 1-1: General method for alkylation of cyclosporin A

Tetrahydrofuran (THF) was added with diisopropyl amine ((i-Pr)$_2$NH) and added with a solution of n-butyl lithium (BuLi) in hexane under a nitrogen atmosphere at -78 °C, followed by stirring for 30 min. To the solution of LDA (lithium diisopropylamide) thus prepared, cyclosporin A in THF was added, stirred for 1 hr, and electrophile was added.

Step 1-2: Synthesis of [D-MeAla^3] cyclosporin A

According to the general method, alkylation was performed employing THF (120 ml), (i-Pr)$_2$NH (1.74 ml), BuLi (4.6 ml), cyclosporin A (2.0 g) in 30 ml THF, and methyliodide (MeI) (0.51 ml). The solution was warmed to room temperature while stirring for 1 hr, and added with 10 ml water, followed by concentration. The residue was added with ether (Et_2O), washed with water and a saturated aqueous sodium chloride solution in sequence, and dried over anhydrous $MgSO_4$. After concentrating, the residue was subjected to silica gel column chromatography (100 g silica gel, dichloromethane : methylalcohol = 50 : 1 ~ 96 : 4), followed by HPLC to give the title compound (0.26 g).

Step 1-3: Preparation of [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A

Herein is described a method of preparing [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A, which retains a hair restoring effect after modification by microorganisms. As a bacterial strain for preparing the cyclosporin derivative, *Sebekia benihana* KCTC 9173 was used. The culture medium contained 0.7 %

glucose, 0.45 % yeast extract, 0.5 % malt extract, 1.0 % soluble starch, and 0.005 % $CaCO_3$, and the incubation temperature was 27℃ (J. Antibiotics, 1996, 49:781-787). After being first pre-cultured for 4 days in an Erlenmeyer flask, the bacterial strain was cultured in the above medium using a 4 L fermentor.

Then, [D-MeAla^3] cyclosporin A in methanol was added at 100 mg/L to the culture medium 24 hrs after starting the main culture. The bacteria were further cultured for 72 hrs.

After 72 hrs, the total culture solution was extracted with an equal volume of ethyl acetate, thereby collecting the sample. The organic solvent layer was concentrated. The concentrated sample was subjected to liquid chromatograhy, isolating and collecting cyclosporin derivatives. The results of liquid chromatography are represented in Fig. 1, which shows [D-MeAla^3] cyclosporin A and [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A derivatives (having a retention time of 18 to 20 min). The isolation condition was as follows. A C-18 column was used. For the elution, a 100 % solvent A was flowed for 2 min. The concentration of the solvent was lowered to 60 %, and the 60 % solvent was flowed over 4 min, and then the concentration was slowly lowered to 39 %, over 60 min. The concentration of the solvent was then returned to 100 %, and flowed for a further 5 min. The solvent A was a 25 % aqueous methanol solution. As the diluent solvent B, 100 % acetonitrile was used.

Molecular weight of the compound was determined by FAB MS (ZMS AX 505H) analysis. To confirm the molecular structure, Nuclear Magnetic Resonance (NMR) measurements were performed on 600 MHz (Bruker) for ^{1}H-NMR and on 150 MHz (Bruker) for ^{13}C-NMR, and the spectra are shown in Figs. 2 and 3, respectively.

Example 2

Preparation of [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

Step 2-1: Synthesis of acetylcyclosporin A

2.4 g (2.0 mmol) of cyclosporin A and 0.24 g (2.0 mmol) of 4-(dimethylamino)pyridine were added to 20 ml of pyridine and 20 ml of acetic anhydride, stirred for 18 hours at room temperature, and distilled under reduced pressure. To the residue was added 100 ml of methylene chloride. The residue

was then washed with water and dried over anhydrous magnesium sulfate ($MgSO_4$). The crude product was purified by chromatography on a silica gel column to give 2.3 g of the title compound.

Step 2-2: Synthesis of acetylcyclosporin A 7-thioamide

1.8 g (1.45 mmol) of acetylcyclosporin was dissolved in 50 ml of xylene. The resulting solution was heated to 130°C, and 0.35 g (0.87 mmol) of Lawesson reagent was added in small amounts thereto. The reaction solution was stirred for 30 minutes at 130°C, cooled to room temperature, and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over anhydrous magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column to give 0.19 g of an acetoxy compound, i.e. the title compound.

Step 2-3: Synthesis of cyclosporin A 7-thioamide

0.2 g (0.16 mmol) of acetylcyclosporin A7-thioamide, the acetoxy compound, was dissolved in 50 ml of methyl alcohol (MeOH). The solution was added with sodium methoxide (NaOMe in MeOH) and stirred for 4 hrs at room temperature. After neutralizing with acetic acid, the resulting solution was distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over anhydrous magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column to give 0.17 g of the title compound.

Step 2-4: Preparation of [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

The bacterial strain used for preparing the cyclosporin derivative was *Sebekia benihana* KCTC 9173. The culture medium contained 0.7 % glucose, 0.45 % yeast extract, 0.5 % malt extract, 1.0 % soluble starch, and 0.005 % $CaCO_3$, and the incubation temperature was 27℃ (J. Antibiotics, 1996, 49:781-787). After being first pre-cultured for 4 days in an Erlenmeyer flask, the bacterial strain was cultured in the above medium using a 4 L fermentor.

Then, cyclosporin A 7-thioamide in methanol was added at 100 mg/L to

the culture medium 24 hrs after starting the main culture. The bacteria were further cultured for 72 hrs.

After 72 hrs, the total culture solution was extracted with an equal volume of ethyl acetate, thereby collecting the sample. The organic solvent layer was concentrated. The concentrated sample was subjected to liquid chromatograhy, isolating and collecting cyclosporin derivatives. The results of liquid chromatography are represented in Fig. 4, which shows cyclosporin A 7-thioamide and [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A (having a retention time of around 16.2 min). The isolation condition was as follows. A C-18 column was used. For the elution, a 100 % solvent A was flowed for 2 min. The concentration of the solvent was lowered to 60 %, and the 60 % solvent was flowed over 4 min, and then the concentration was slowly lowered to 39 %, over 60 min. The concentration of the solvent was then returned to 100 %, and flowed for a further 5 min. The solvent A was a 25 % aqueous methanol solution. As the diluent solvent B, 100 % acetonitrile was used. Molecular weight of the compound was determined by FAB MS (ZMS AX 505H) analysis. To confirm the molecular structure, Nuclear Magnetic Resonance (NMR) measurements were performed on 600 MHz (Bruker) for ^{1}H-NMR and on 150 MHz (Bruker) for ^{13}C-NMR, and the spectra are shown in Figs. 5 and 6, respectively.

Example 3

Preparation of [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A

As a bacterial strain for preparing the cyclosporin derivative, *Pseudonocardia autotrophica* KCTC 9441 was used. The culture medium contained 0.7 % glucose, 0.45 % yeast extract, 0.5 % malt extract, 1.0 % soluble starch, and 0.005 % $CaCO_3$, and the incubation temperature was 27℃ (J. Antibiotics, 1996, 49: 781-787). After being first pre-cultured for 4 days in an Erlenmeyer flask, the bacterial strain was cultured in the above medium using a 4 L fermentor. Then, cyclosporin A in methanol was added at 100 mg/L to the culture medium 24 hrs after starting the main culture. The bacteria were further cultured for 72 hrs.

After 72 hrs, the total culture solution was extracted with an equal volume

of ethyl acetate, thereby collecting the sample. The organic solvent layer was concentrated. The concentrated sample was subjected to liquid chromatograhy, isolating and collecting the cyclosporin derivative. The isolation condition was as follows. A C-18 column was used. For the elution, a 100 % solvent A was flowed for 2 min. The concentration of the solvent was lowered to 60 %, and the 60 % solvent was flowed over 4 min, and then the concentration was slowly lowered to 39 %, over 60 min. The concentration of the solvent was then returned to 100 %, and flowed for a further 5 min. The solvent A was a 25 % aqueous methanol solution. As the diluent solvent B, 100 % acetonitrile was used. Molecular weight of the compound was determined by FAB MS (ZMS AX 505H) analysis. To confirm the molecular structure, Nuclear Magnetic Resonance (NMR) measurements were performed on 600 MHz (Bruker) for ^{1}H-NMR and on 150 MHz (Bruker) for ^{13}C-NMR, and the spectra are shown in Figs. 7 and 8, respectively.

Alternatively, [γ-hydroxy-N-methyl-L-leucine^{4}][γ-hydroxy-N-methyl-L-leucine^{9}] cyclosporin A can be prepared using microsomal enzyme obtained from rabbit's liver. First, a liver was removed from a New Zealand White rabbit and immersed in 0.1 M potassium phosphate buffer for 5 min. The liver tissue was minced and homogenized, followed by centrifuging (9000 x g, 4 °C, 20 min). The supernatant was taken and centrifuged again (10,500 x g, 1 hr). After discarding the supernatant, the pellet was dissolved in 0.1 M phosphate buffered saline, to serve as a source of the enzyme (Transplant Proc., 17: 633-636, 1988). The microsomal enzyme (50 mg) thus prepared, cyclosporin (1 mg) and NADPH (5 mM) were mixed with water, making a desired amount of solution. The solution was reacted at 37 °C in water bath for 1 hr. The reaction mixture was extracted with an equal volume of ethyl acetate, followed by analysis.

Example 4

Preparation of [L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A

Step 4-1: Preparation of [L-threonine^{2}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A peptolide

A fungal strain, Cylindrotrichum Bonorden NRRL 18230 was used (Biomed. Biochim. Acta., 10/11, S260-S263, 1991). The culture medium

contained 0.7 % glucose, 0.45 % yeast extract, 0.5 % malt extract, 1.0 % soluble starch, and 0.005 % $CaCO_3$, and the incubation temperature was 27℃. After being first pre-cultured for 4 days in an Erlenmeyer flask, the strain was cultured in the above medium using a 4 L fermentor.

After being cultured, the total culture solution was extracted with an equal volume of ethyl acetate, thereby collecting the sample. The organic solvent layer was concentrated. The concentrated sample was subjected to liquid chromatograhy, isolating and collecting the cyclosporin derivative. The isolation condition was as follows. A C-18 column was used. For the elution, a 100 % solvent A was flowed for 2 min. The concentration of the solvent was lowered to 60 %, and the 60 % solvent was flowed over 4 min, and then the concentration was slowly lowered to 39 %, over 60 min. The concentration of the solvent was then returned to 100 %, and flowed for a further 5 min. The solvent A was a 25 % aqueous methanol solution. As the diluent solvent B, 100 % acetonitrile was used.

Step 4-2: Preparation of [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A

For preparing the cyclosporin derivative, *Sebekia benihana* KCTC 9173 strain was used. The culture medium contains 0.7 % glucose, 0.45 % yeast extract, 0.5 % malt extract, 1.0 % soluble starch, and 0.005 % $CaCO_3$, and the incubation temperature was 27℃ (J. Antibiotics, 1996, 49:781-787). Upon culturing using a fermentor, a pre-culture was first performed for 4 days in an Erlenmeyer flask. After pre-culture, the bacterial strain was cultured in the above medium using a 4 L fermentor.

Then, [L-threonine^2][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A peptolide in methanol was added at 100 mg/L to the culture medium 24 hrs after starting the main culture. The bacteria were further cultured for 72 hrs.

After 72 hrs, the total culture solution was extracted with an equal volume of ethyl acetate, thereby collecting the sample. The organic solvent layer was concentrated. The concentrated sample was subjected to liquid chromatograhy, isolating and collecting the cyclosporin derivative. The isolation condition was as follows. A C-18 column was used. For the elution, a 100 % solvent A was flowed for 2 min. The concentration of the solvent was lowered to 60 %, and the 60 % solvent was flowed over 4 min, and then the concentration was slowly lowered

to 39 %, over 60 min. The concentration of the solvent was then returned to 100 %, and flowed for a further 5 min. The solvent A was a 25 % aqueous methanol solution. As the diluent solvent B, 100 % acetonitrile was used. The molecular weight of [L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A was determined by FAB MS (ZMS AX 505H) analysis. [M (peptolide derivative)+Na] peak was observed at m/z 1286.0 (Fig. 9). To confirm the molecular structure, Nuclear Magnetic Resonance (NMR) measurements were performed on 600 MHz (Bruker) for ^{1}H-NMR and on 150 MHz (Bruker) for ^{13}C-NMR.

FORMULATIONS

FORMULATION 1-1:

Preparation of hair tonic containing [N-methyl-D-alanine^{3}][γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin A

Individual ingredients were mixed and stirred, and the mixtures were completely dissolved to prepare three hair growth promoting tonics, with compositions as shown in Table 1 below. It was found that the composition 1 of Table 1 has a hair growth promoting effect at a level similar to a conventional hair tonic containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 1: Formulation of hair tonic

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
ethanol	40.0	40.0	40.0
[N-methyl-D-alanine^{3}][γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin A	0.1	1.0	8.0
tocopherol acetate	0.1	0.1	0.1
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 1-2:

Preparation of hair tonic containing [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

Individual ingredients were mixed and stirred, and the mixtures were completely dissolved to prepare three hair growth promoting tonics, with compositions as shown in Table 2 below. It was found that the composition 1 of Table 2 has a hair growth promoting effect at a level similar to a conventional hair tonic containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 2: Formulation of hair tonic

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
ethanol	40.0	40.0	40.0
[γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A	0.1	1.0	8.0
tocopherol acetate	0.1	0.1	0.1
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 1-3:

Preparation of hair tonic containing [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A

Individual ingredients were mixed and stirred, and the mixtures were completely dissolved to prepare three hair growth promoting tonics, with compositions as shown in Table 3 below. It was found that the composition 1 of Table 3 has a hair growth promoting effect at a level similar to a conventional hair tonic containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 3: Formulation of hair tonic

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
ethanol	40.0	40.0	40.0
[γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A	0.1	1.0	8.0
tocopherol acetate	0.1	0.1	0.1
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 1-4:

Preparation of hair tonic containing [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A

Individual ingredients were mixed and stirred, and the mixtures were completely dissolved to prepare three hair growth promoting tonics, with compositions as shown in Table 4 below. It was found that the composition 1 of Table 4 has a hair growth promoting effect at a level similar to a conventional hair tonic containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 4: Formulation of hair tonic

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
ethanol	40.0	40.0	40.0
[L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A	0.1	1.0	8.0
tocopherol acetate	0.1	0.1	0.1
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5

perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 2-1:

Preparation of hair cream containing [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair creams, with compositions as shown in Table 5 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

It was found that the composition 1 of Table 5 has a hair growth promoting effect at a level similar to a conventional hair cream containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 5: Formulation of hair cream

(unit: weight %)

Ingredients	Comp: 1	Comp. 2	Comp. 3
paraffin	5.0	5.0	5.0
cetostearyl alcohol	5.5	5.5	5.5
petrolatum	5.5	5.5	5.5
glycerin monostearate	3.0	3.0	3.0
polyoxyethyleneoctyldodecylether	3.0	3.0	3.0
propylparaben	0.3	0.3	0.3
[N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A	0.1	1.0	8.0
glycerin	7.0	7.0	7.0
dipropyleneglycol	20.0	20.0	20.0
polyethyleneglycol	5.0	5.0	5.0
water	balance not including perfume and colorant		
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 2-2:

Preparation of hair cream containing [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair creams, with compositions as shown in Table 6 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

It was found that the composition 1 of Table 6 has a hair growth promoting effect at a level similar to a conventional hair cream containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 6: Formulation of hair cream

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
paraffin	5.0	5.0	5.0
cetostearyl alcohol	5.5	5.5	5.5
petrolatum	5.5	5.5	5.5
glycerin monostearate	3.0	3.0	3.0
polyoxyethyleneoctyldodecylether	3.0	3.0	3.0
propylparaben	0.3	0.3	0.3
[γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A	0.1	1.0	8.0
glycerin	7.0	7.0	7.0
dipropyleneglycol	20.0	20.0	20.0
polyethyleneglycol	5.0	5.0	5.0
water	balance not including perfume and colorant		
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 2-3:

Preparation of hair cream containing [γ-hydroxy-N-methyl-L-leucine^4][γ-

hydroxy-N-methyl-L-leucine[9]] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair creams, with compositions as shown in Table 7 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

It was found that the composition 1 of Table 7 has a hair growth promoting effect at a level similar to a conventional hair cream containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later

Table 7: Formulation of hair cream

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
paraffin	5.0	5.0	5.0
cetostearyl alcohol	5.5	5.5	5.5
petrolatum	5.5	5.5	5.5
glycerin monostearate	3.0	3.0	3.0
polyoxyethyleneoctyldodecylether	3.0	3.0	3.0
propylparaben	0.3	0.3	0.3
[γ-hydroxy-N-methyl-L-leucine[4]][γ-hydroxy-N-methyl-L-leucine[9]] cyclosporin A	0.1	1.0	8.0
glycerin	7.0	7.0	7.0
dipropyleneglycol	20.0	20.0	20.0
polyethyleneglycol	5.0	5.0	5.0
water	balance not including perfume and colorant		
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 2-4:

Preparation of hair cream containing [L-threonine[2]][γ-hydroxy-N-methyl-L-leucine[4]][L-leucine[5]][D-2-hydroxyisovaleric acid[8]][L-leucine[10]] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two

phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair creams, with compositions as shown in Table 8 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

It was found that the composition 1 of Table 8 has a hair growth promoting effect at a level similar to a conventional hair cream containing 0.1 % cyclosporin A, as evaluated in an animal experiment according to Test Example 1 described later.

Table 8: Formulation of hair cream

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
paraffin	5.0	5.0	5.0
cetostearyl alcohol	5.5	5.5	5.5
petrolatum	5.5	5.5	5.5
glycerin monostearate	3.0	3.0	3.0
polyoxyethyleneoctyldodecylether	3.0	3.0	3.0
propylparaben	0.3	0.3	0.3
[L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A	0.1	1.0	8.0
glycerin	7.0	7.0	7.0
dipropyleneglycol	20.0	20.0	20.0
polyethyleneglycol	5.0	5.0	5.0
water	balance not including perfume and colorant		
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 3-1:

Preparation of shampoo containing [N-methyl-D-alanine^{3}][γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin A

All individual ingredients, except perfume, colorant and water, were mixed and the mixture was completely dissolved by heating, while stirring. After

cooling to room temperature, the mixture was mixed with perfume and colorant. Water was finally added to adjust to 100 % the total weight, to prepare three shampoos, with compositions as shown in Table 9 below.

Table 9: Formulation of shampoo

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
palm oil fattyacid diethanolamide	3.0	3.0	3.0
propyleneglycol	2.0	2.0	2.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
ehtanol	2.0	2.0	2.0
[N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A	1.0	3.0	10.0
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 3-2:

Preparation of shampoo containing [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

All individual ingredients, except perfume, colorant and water, were mixed and the mixture was completely dissolved by heating, while stirring. After cooling to room temperature, the mixture was mixed with perfume and colorant. Water was finally added to adjust to 100 % the total weight, to prepare three shampoos, with compositions as shown in Table 10 below.

Table 10: Formulation of shampoo

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0

palm oil fattyacid diethanolamide	3.0	3.0	3.0
propyleneglycol	2.0	2.0	2.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
ehtanol	2.0	2.0	2.0
[γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A	1.0	3.0	10.0
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 3-3:

Preparation of shampoo containing [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A

All individual ingredients, except perfume, colorant and water, were mixed and the mixture was completely dissolved by heating, while stirring. After cooling to room temperature, the mixture was mixed with perfume and colorant. Water was finally added to adjust to 100 % the total weight, to prepare three shampoos, with compositions as shown in Table 11 below.

Table 11: Formulation of shampoo

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
palm oil fattyacid diethanolamide	3.0	3.0	3.0
propyleneglycol	2.0	2.0	2.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
ehtanol	2.0	2.0	2.0
[γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A	1.0	3.0	10.0
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
perfume	typical	typical	typical

colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 3-4:

Preparation of shampoo containing [L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A

All individual ingredients, except perfume, colorant and water, were mixed and the mixture was completely dissolved by heating, while stirring. After cooling to room temperature, the mixture was mixed with perfume and colorant. Water was finally added to adjust to 100 % the total weight, to prepare three shampoos, with compositions as shown in Table 12 below.

Table 12: Formulation of shampoo

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
palm oil fattyacid diethanolamide	3.0	3.0	3.0
propyleneglycol	2.0	2.0	2.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
ehtanol	2.0	2.0	2.0
[L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A	1.0	3.0	10.0
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
perfume	typical	typical	typical
colorant	typical	typical	typical
water	balance	balance	balance

FORMULATION 4-1:

Preparation of hair conditioner containing [N-methyl-D-alanine^{3}][γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two

phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair conditioners, with compositions as shown in Table 13 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

Table 13: Formulation of hair conditioner

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
cetanol	3.0	3.0	3.0
self-emulsifiable glycerol-monostearate	2.0	2.0	3.0
squalene	10.0	10.0	10.0
[N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A	1.0	5.0	10.0
propyleneglycol	2.0	2.0	2.0
stearyldimethyl benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
water	balance	balance	balance
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 4-2:

Preparation of hair conditioner containing [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair conditioners, with compositions as shown in Table 14 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

Table 14: Formulation of hair conditioner

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
cetanol	3.0	3.0	3.0
self-emulsifiable glycerol-monostearate	2.0	2.0	3.0
squalene	10.0	10.0	10.0
[γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A	1.0	5.0	10.0
propyleneglycol	2.0	2.0	2.0
stearyldimethyl benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
water	balance	balance	balance
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 4-3:

Preparation of hair conditioner containing [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair conditioners, with compositions as shown in Table 15 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

Table 15: Formulation of hair conditioner

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
cetanol	3.0	3.0	3.0
self-emulsifiable glycerol-monostearate	2.0	2.0	3.0
squalene	10.0	10.0	10.0

[γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A	1.0	5.0	10.0
propyleneglycol	2.0	2.0	2.0
stearyldimethyl benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
methyl paraoxybenzoic acid	0.2	0.2	0.2
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
water	balance	balance	balance
perfume	typical	typical	typical
colorant	typical	typical	typical

FORMULATION 4-4:

Preparation of hair conditioner containing [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A

Individual oil-phase and water-phase ingredients were mixed in a separate container, and each mixture was completely dissolved by heating to 80 °C. Two phases of the ingredients were mixed, emulsified, and cooled to room temperature. Additives such as perfume and colorant were admixed to prepare three hair conditioners, with compositions as shown in Table 16 below. Water was added to adjust to 100 % the total weight including the oil-phase and water-phase ingredients.

Table 16: Formulation of hair conditioner

(unit: weight %)

Ingredients	Comp. 1	Comp. 2	Comp. 3
cetanol	3.0	3.0	3.0
self-emulsifiable glycerol-monostearate	2.0	2.0	3.0
squalene	10.0	10.0	10.0
[L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A	1.0	5.0	10.0
propyleneglycol	2.0	2.0	2.0
stearyldimethyl benzylammonium chloride	8.0	8.0	8.0

(25 wt% aqueous solution)			
methyl paraoxybenzoic acid	0.2	0.2	0.2
salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
water	balance	balance	balance
perfume	typical	typical	typical
colorant	typical	typical	typical

Test Example 1: Evaluation of hair restoring effect in mice

Female C57BL/6 mice of ages 6 to 7 weeks were utilized. After removing hairs on the middle of the back with an electric shaver, the mice were weighed and randomly assigned to the test groups with an even distribution of weights. After one day of adaptation, the mice were applied with cyclosporin A and the cyclosporin A derivatives prepared by HPLC in Examples 1 to 4 to their hair removed areas once a day at a dose of 100 $\mu\ell$ (conc. 0.1 % w/v), for 30 days. The results were determined by visual examination, in terms of degrees of hair regrowth. With respect to respective hair-removed areas, rates of new hair growth were examined and compared.

As can be seen in Table 17, cyclosporin derivatives of the invention have a significant hair growth promoting effect, compared to the control in which mice were applied with a vehicle only. Further, the derivatives show a similar level of hair growth promoting effect, with respect to cyclosporin A. Meanwhile, over the course of 30 days, as comparing the appearance of the backs, the mice of the control and all test groups showed no specific skin irritation.

Table 17: Hair restore in mice

Compound applied	Area rate of hair regrowth (%)
vehicle	35
cyclosporin A	91
[N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A	95
[γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A	91

[γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A	85
[L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A	90

Test Example 2

Evaluation of non-immunosuppressive activity *in vitro*

To compare immunosuppressive activities of cyclosporin derivatives according to the invention with that of cyclosporin A, the MLR method (Mixed Lymphocyte Reaction method, J. Antibiotics, 1994, 47:208-215), employing splenocytes obtained from two distinct species of mice, was used.

Responder cells from BALB/c mice were mixed with an equal number of stimulator cells from C57BL/6 mice pre-treated with mitomycin. The mixed splenocytes were respectively treated with cyclosporin A and the cyclosporin derivatives of the invention, and incubated in RPMI media (supplemented with mercaptoethanol and 10 % fetal bovine serum) for 4 days. After 4 days of incubation, ^{3}H-thymidine was added to the mixtures and incubated for an additional 4 hours. Then, the amount of thymidine incorporated into the cells was measured (liquid scintillation counter), and IC_{50} (μg/ml) of respective cyclosporins were calculated.

As a result, IC_{50} (μg/ml) of cyclosporin A was 0.022, 0.042 and 0.040 in triplicate cultures, respectively. On the other hand, the IC_{50} (μg/ml) values for [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A were 8.2, 8.8 and 9.3, for [γ-hydroxy-N-methyl-L-leucine^4] [alanine thiomide^7, $^7\psi^8$ CS-NH] cyclosporin A, 10.1, 13.2 and 13.9, for γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A, 18.2, 19.2 and 17.2, and for [L-threonine^2][γ-hydroxy-N-methyl-L-leucine^4][L-leucine^5][D-2-hydroxyisovaleric acid^8][L-leucine^{10}] cyclosporin A, 30.1, 29.0 and 19.2, indicating more than 100-fold decrease in its immunosuppressive activity, compared to cyclosporin A.

These findings prove that [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin derivatives which are added with a hydroxyl group by microbial metabolism to gamma carbon in N-methyl-L-leucine, the residue No. 4 of cyclosporin, have very weak immunosuppressive activities, compared to unmodified cyclosporin A, while they retain an excellent hair growth effect.

On the basis of the foregoing results, the cyclosporin derivatives of the invention may be formulated in any form including liquid formulations, sprays, gels, pastes, emulsions, creams, conditioners, shampoos, and the like. A variety of forms are available though, considering their high commercial demand, hair tonics, creams, conditioners, and shampoos are provided herein. As revealed in the above the Test Example 1, the cyclosporin derivatives exhibit an excellent hair growth promoting effect, compared to the control.

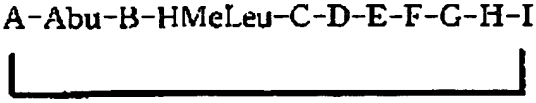
Industrial Applicability

As apparent from the above description, the present invention provides a hair growth promoting agent, comprising a cyclosporin derivative as an active ingredient, exhibits an excellent hair growth effect, while it shows a very weak immunosuppressive activity, compared to unmodified cyclosporin A.

Claims

1. A hair growth promoting agent comprising a [γ-hydroxy-N-methyl-L-leucine[4]] cyclosporin derivative represented by Chemical Formula 1 below, having an excellent hair restoring effect with non-immunosuppressive activity, as an active ingredient:

[Chemical Formula 1]



in which

A represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine (MeBmt), (2S,3R,4R,6E)-3-sulfhydryl-4-methyl-2-(methylamino)-6-octenoic acid or (2S,4R,6E)-3-oxo-4-methyl-2-(methylamino)-6-octenoic acid;

Abu represents L-α-aminobutyric acid (Abu);

B represents a D-amino acid represented by the general formula 1,

[General formula 1]

CH_3NH-CH(R)-COOH

in which,

R is one selected from the group consisting of hydrogen, C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino, and X-R' represented by the general formula 2 below,

[General formula 2]

-X-R'

in which,

X is oxygen or sulfur, and

R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

C represents L-valine or L-norvaline;

D represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine;

E represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

F represents D-2-hydroxyisovaleric acid or a D-amino acid represented by the general formula 3,

[General formula 3]

-NH-CH(CH$_2$R)-COOH

in which,

R is hydrogen or X-R' represented by the general formula 4,

[General formula 4]

-X-R'

in which,

X is oxygen or sulfur, and

R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

G represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine;

H represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

I represents N-methyl-L-valine or L-valine,

excepted that if B is sarcosine, then C is L-valine, D is N-methyl-L-leucine, E is L-alanine, F is D-alanine, G is N-methyl-L-leucine, H is N-methyl-L-leucine, and I is N-methyl-L-valine.

2. The hair growth promoting agent as set forth in claim 1, wherein the [γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin derivative is represented by Chemical Formula 2:

[Chemical Formula 2]

MeBmt-Abu-A'-HMeLeu-Val-MeLeu-B'-C'-D'-MeLeu-MeVal

in which

MeBmt represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine;

Abu represents L-α-aminobutyric acid (Abu);

A' represents N-methyl-D-alanine, D-2-(methylamino)pent-4-enoyl, N-methyl-D-aminobutyric acid, N-methyl-D-norvaline, D-2-(methylamino)hexa-4-ynoyl, D-2-(methylamino)pent-4-ynoyl, D-2-methylthio-sarcosine, N-methyl-O-propenyl-D-serine, N-methyl-D-serine or sarcosine;

HMeLeu represents γ-hydroxy-N-methyl-L-leucine;

Val represents L-valine;

MeLeu represents N-methyl-L-leucine;

B' represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

C' represents D-2-hydroxyisovaleric acid, or a D-amino acid represented by the general formula 3,

[General formula 3]

-NH-CH(CH_2R)-COOH

in which,

R is hydrogen or X-R' represented by the general formula 4,

[General formula 4]

-X-R'

in which,

X is oxygen or sulfur, and

R' is one selected from the group consisting of hydrogen, and C_1-C_6 straight or branched alkyl, alkenyl or alkynyl moieties, substituted or unsubstituted with one or more selected from the group consisting of amino, hydroxy, halo, haloalkyl, ester, alkoxy, cyano, nitro, alkylamino, and dialkylamino;

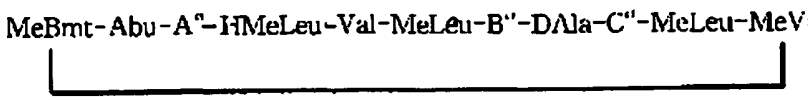
D' represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

MeVal represents N-methyl-L-valine,

excepted that if A is sarcosine, then B is L-alanine, C is D-alanine, and D is N-methyl-L-leucine.

3. The hair growth promoting agent as set forth in claim 1, wherein the [γ-hydroxy-N-methyl-L-leucine^{4}] cyclosporin derivative is represented by Chemical Formula 3:

[Chemical Formula 3]



in which

MeBmt represents N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine;

Abu represents L-α-aminobutyric acid (Abu);

A" represents N-methyl-D-alanine, D-2-(methylamino)pent-4-enoyl, N-methyl-D-aminobutyric acid, N-methyl-D-norvaline, D-2-(methylamino)hexa-4-ynoyl, D-2-(methylamino)pent-4-ynoyl, D-2-methylthio-sarcosine, N-methyl-O-propenyl-D-serine, N-methyl-D-serine or sarcosine;

HMeLeu represents γ-hydroxy-N-methyl-L-leucine;

Val represents L-valine;

MeLeu represents N-methyl-L-leucine;

B" represents L-alanine or L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-CHCH$_3$-CS-);

DAla represents D-alanine;

C" represents N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine or L-leucine; and

MeVal represents N-methyl-L-valine,

excepted that if A is sarcosine, then B is L-alanine, and C is N-methyl-L-leucine.

4. The hair growth promoting agent as set forth in claim 3, comprising [N-methyl-D-alanine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

5. The hair growth promoting agent as set forth in claim 3, comprising [D-2-(methylamino)pent-4-enoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

6. The hair growth promoting agent as set forth in claim 3, comprising [N-methyl-D-Abu^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

7. The hair growth promoting agent as set forth in claim 3, comprising [N-methyl-D-Norvaline^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

8. The hair growth promoting agent as set forth in claim 3, comprising [D-2-(methylamino)hexa-4-ynoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

9. The hair growth promoting agent as set forth in claim 3, comprising [D-2-(methylamino)pent-4-ynoyl^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

10. The hair growth promoting agent as set forth in claim 3, comprising [D-2-methylamino-Sar^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

11. The hair growth promoting agent as set forth in claim 3, comprising [N-methyl-O-propenyl-D-serine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

12. The hair growth promoting agent as set forth in claim 3, comprising [N-methyl-D-serine^3][γ-hydroxy-N-methyl-L-leucine^4] cyclosporin A as an active ingredient.

13. The hair growth promoting agent as set forth in claim 3, comprising [γ-hydroxy-N-methyl-L-leucine^4][alanine thioamide^7, $^7\psi^8$ CS-NH] cyclosporin A as an active ingredient.

14. The hair growth promoting agent as set forth in claim 3, comprising [γ-hydroxy-N-methyl-L-leucine^4][γ-hydroxy-N-methyl-L-leucine^9] cyclosporin A as an active ingredient.

15. The hair growth promoting agent as set forth in claim 3, comprising [γ-hydroxy-N-methyl-L-leucine^4][D-serine^8] cyclosporin A as an active ingredient.

16. The hair growth promoting agent as set forth in claim 3, comprising [L-threonine^{2}][γ-hydroxy-N-methyl-L-leucine^{4}][L-leucine^{5}][D-2-hydroxyisovaleric acid^{8}][L-leucine^{10}] cyclosporin A as an active ingredient.

17. The hair growth promoting agent as set forth in any one of claims 1 to 16, which is formulated in a form selected from the group consisting of liquid formulation, spray, gel, paste, emulsion, cream, conditioner and shampoo.

Fig.1

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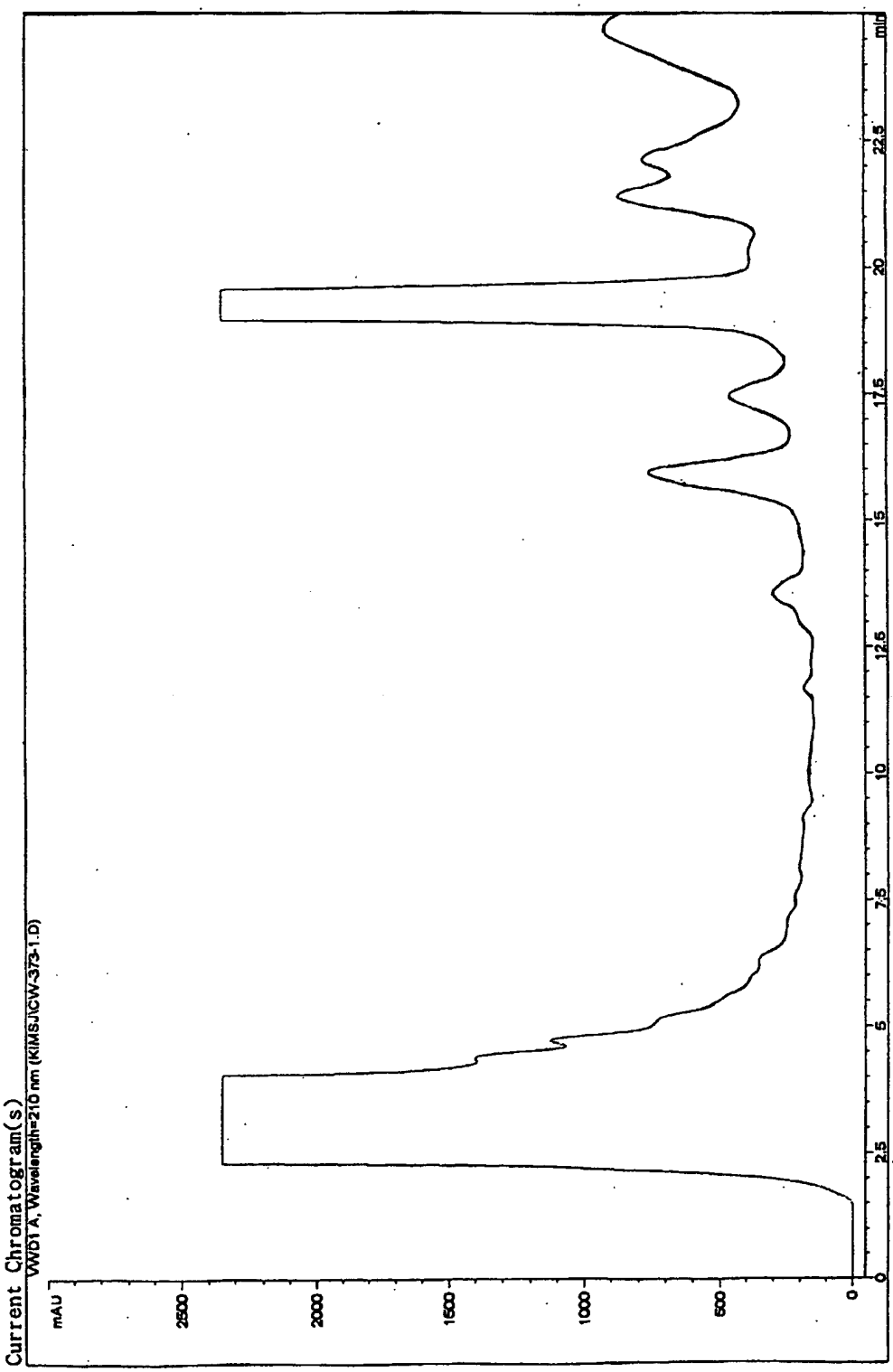

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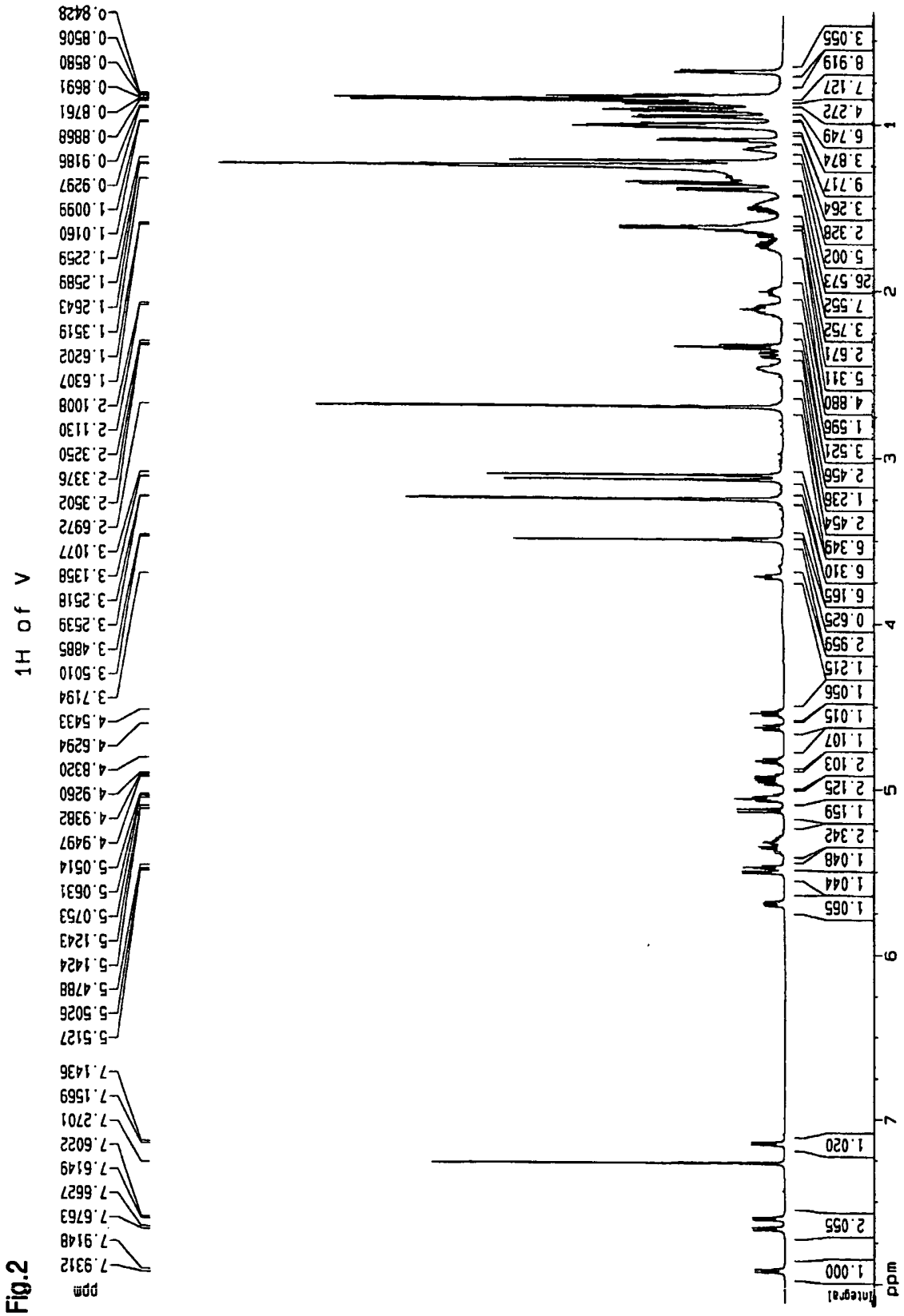
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7.127
4.272
6.749
3.874
9.717
3.264
2.328
5.002
26.573
7.552
3.752
2.671
5.311
4.880
1.596
3.521
2.456
1.238
2.454
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6.310
6.165
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1.056
1.015
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2.055
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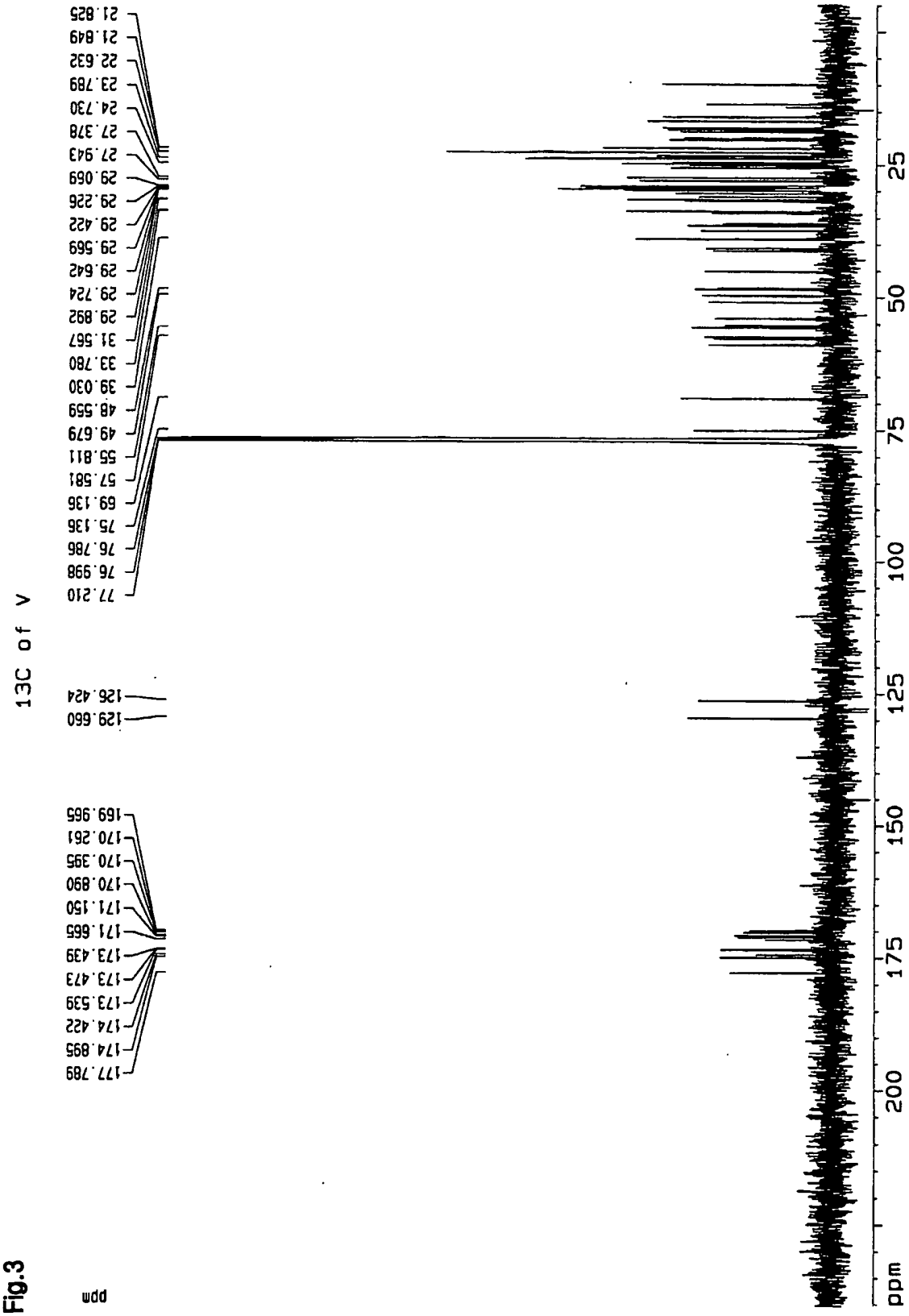
Fig.2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29.642
29.724
29.892
31.567
33.780
39.030
48.559
49.679
55.811
57.581
69.136
75.136
76.786
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171.150
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173.539
174.422
174.895
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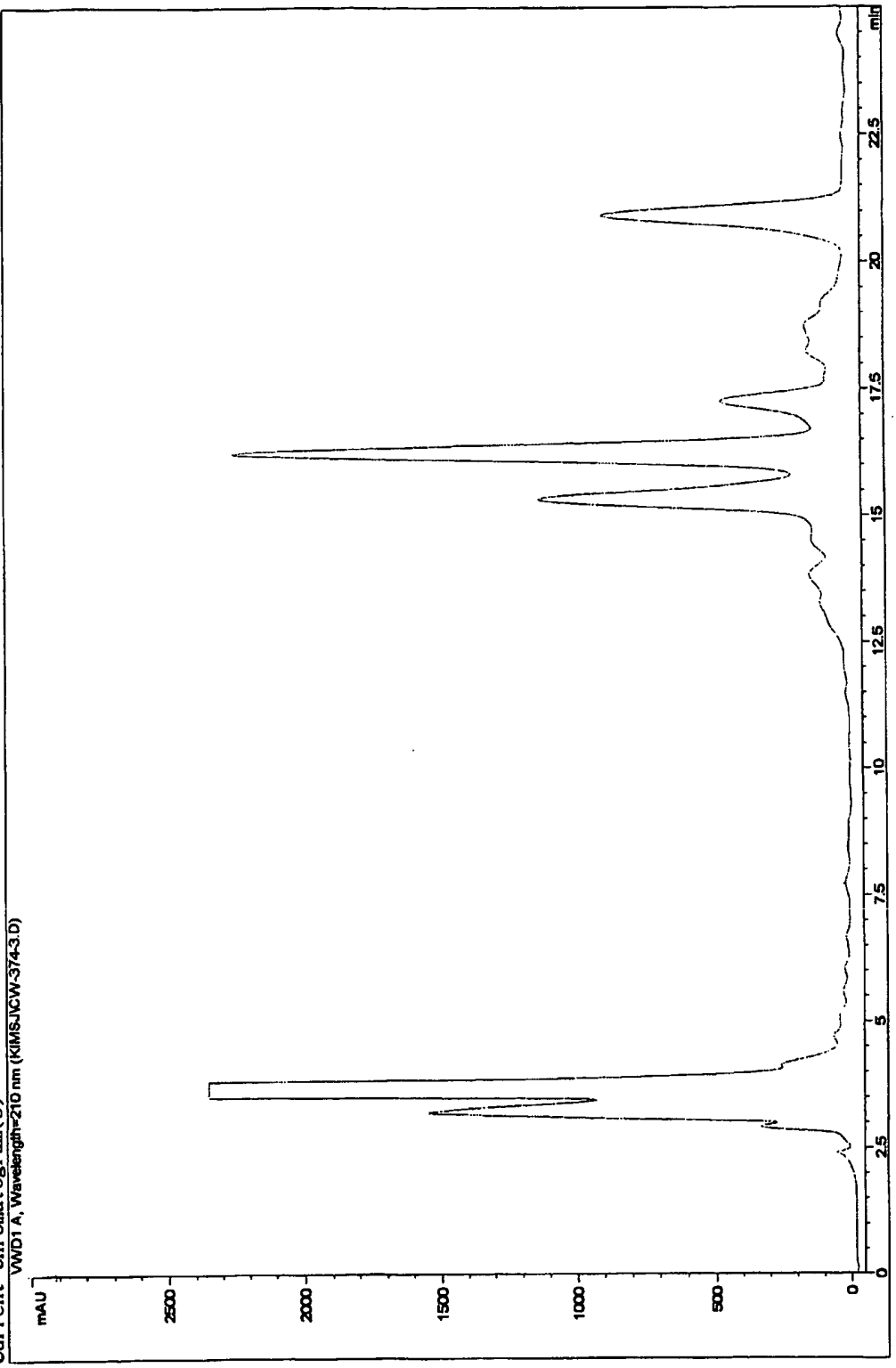
75
100
125
150
175
200
ppm

Fig.3

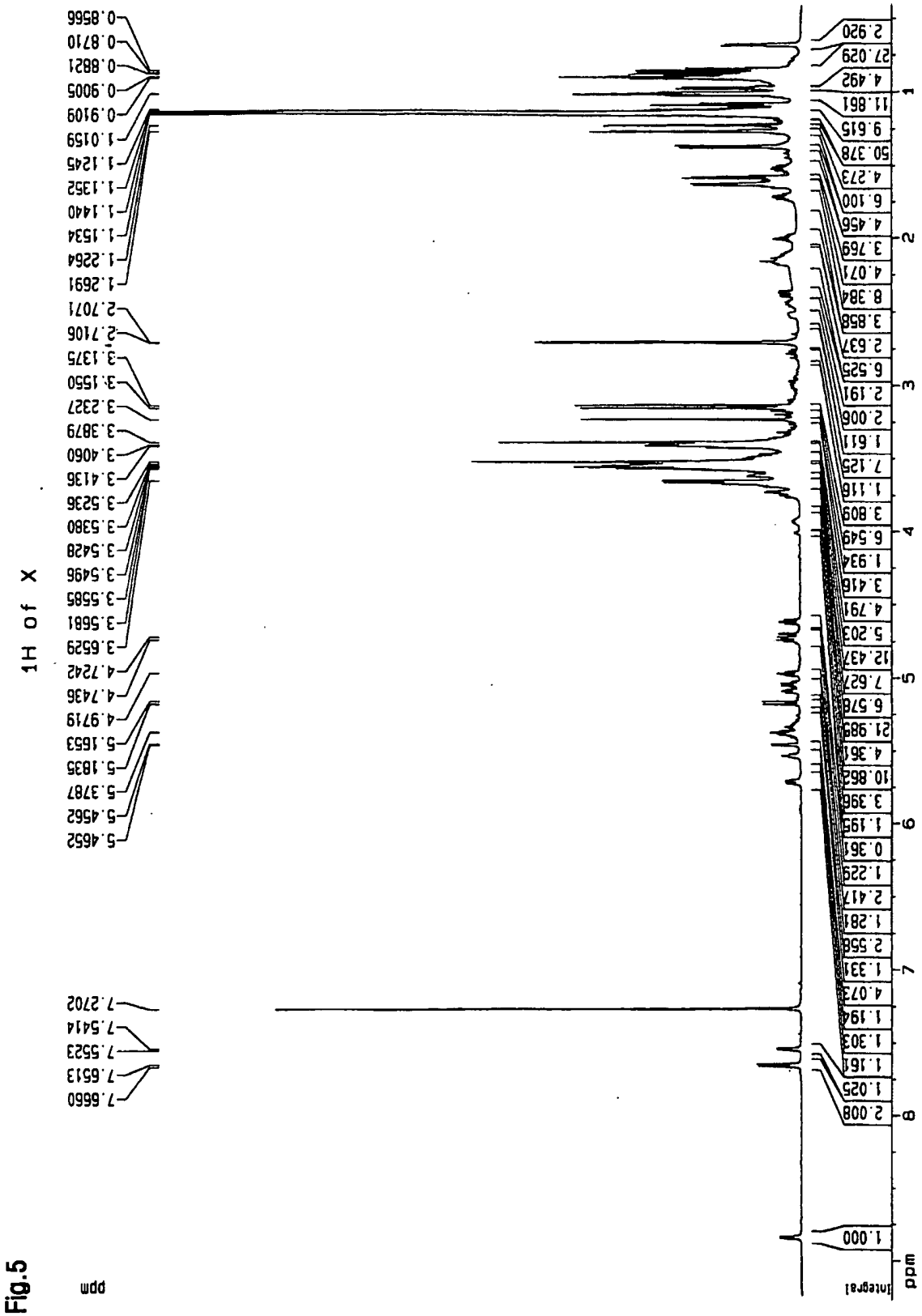
Fig.4

Print of window 38: Current Chromatogram(s)



Instrument 1 5/15/01 10:52:46 AM jhkim

Page 1 of 1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1.2691
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3.1550
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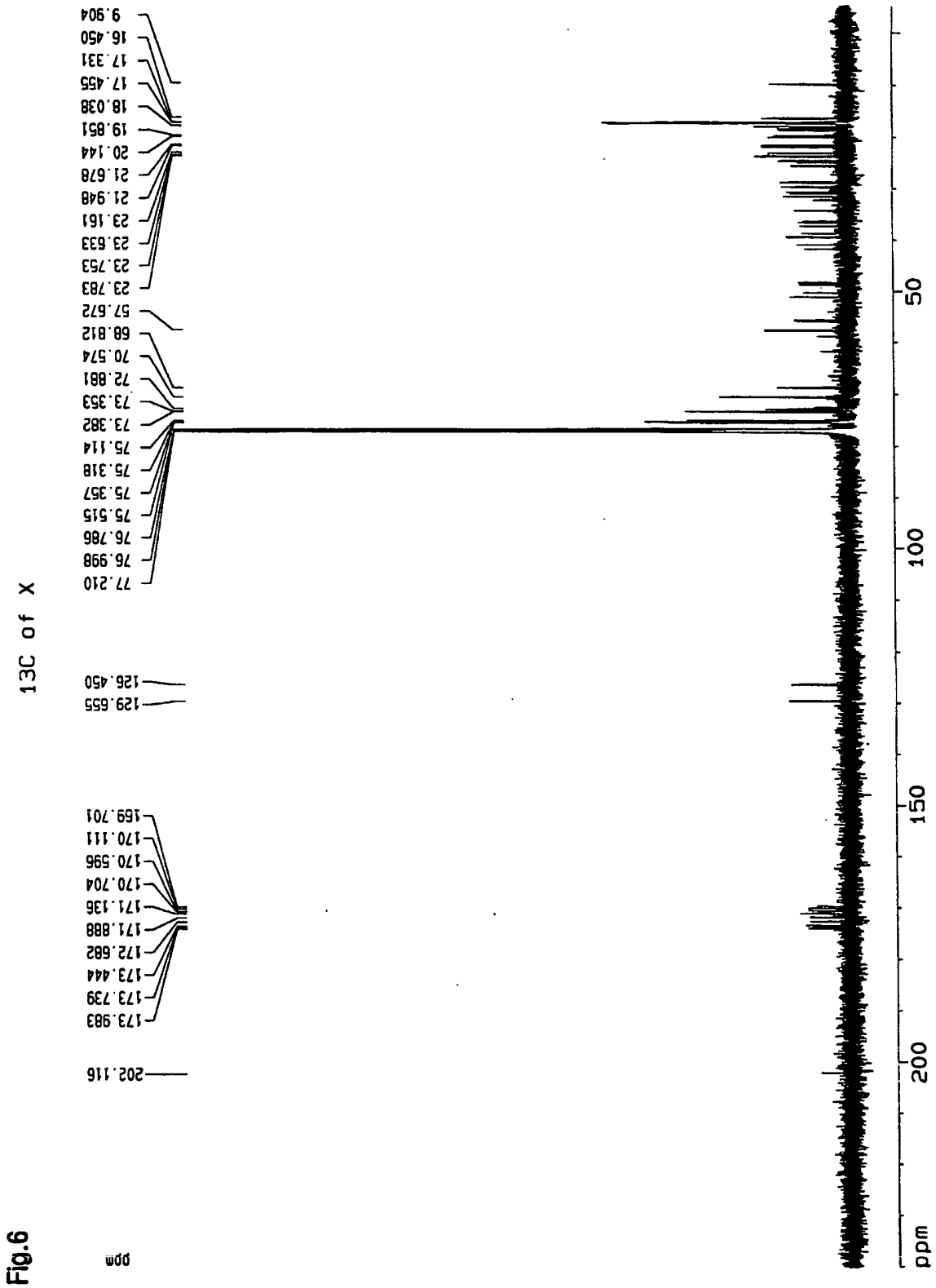
1.025
2.008
1.000

Fig.5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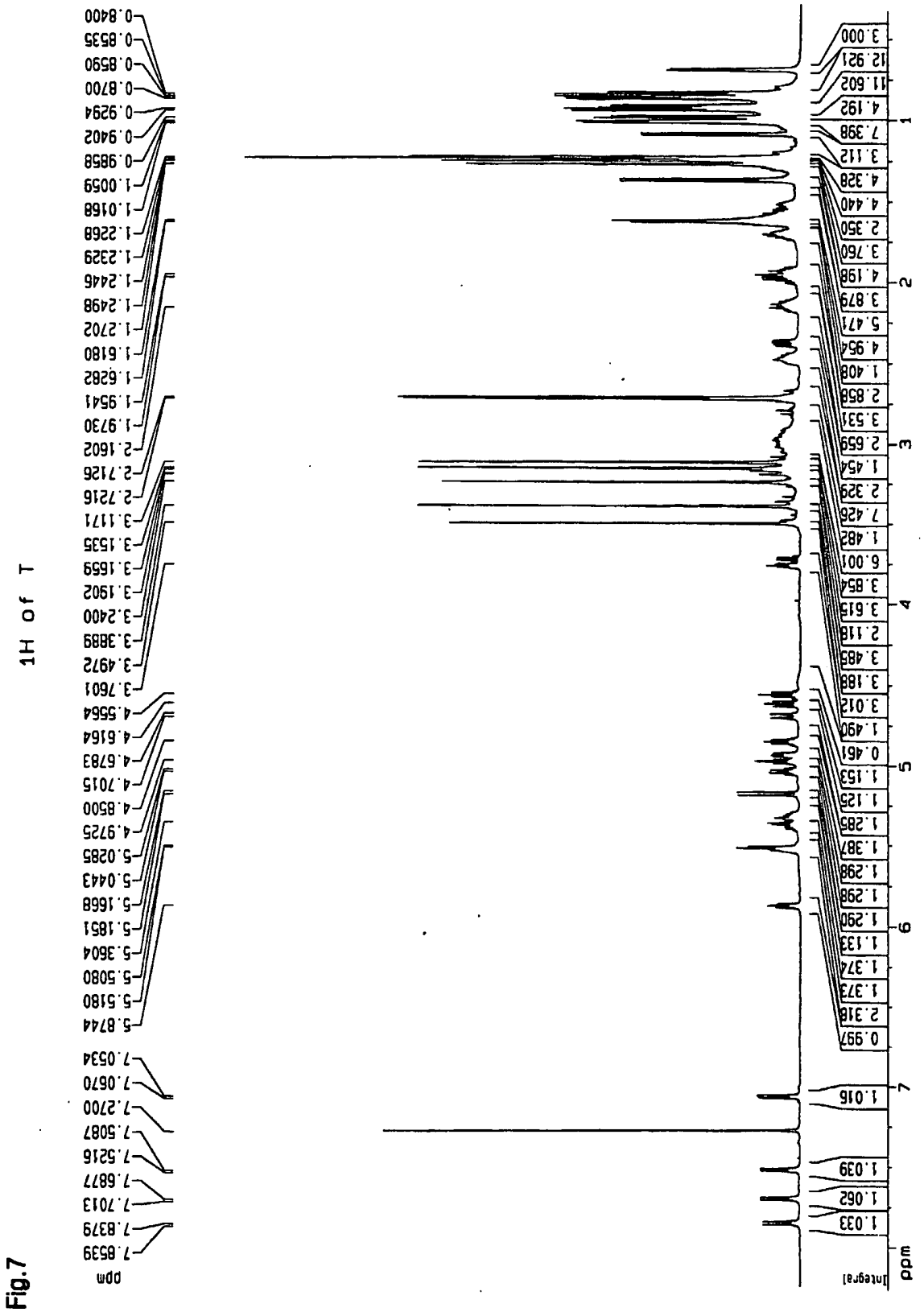
150
200
ppm

Fig.6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7.2700
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5.1851
5.1668
5.0443
5.0285
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4.8500
4.7015
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3.7601
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3.2400
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3.1659
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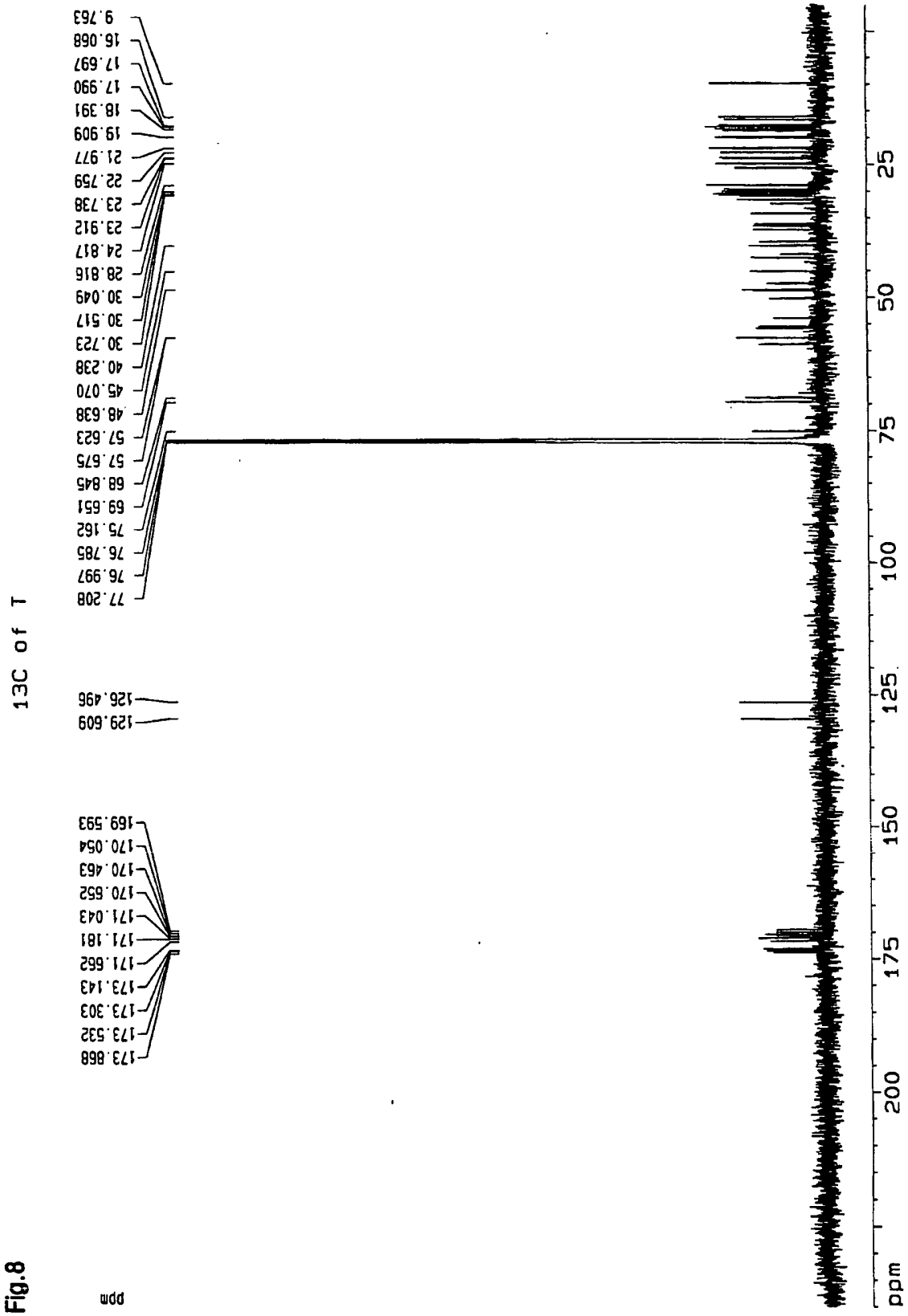
Fig.7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45.070
48.638
57.623
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75.162
76.785
76.997
77.208
126.496
129.609
169.593
170.054
170.463
170.652
171.043
171.181
171.662
173.143
173.303
173.532
173.868
13C of T
ppm
25
50
75
100
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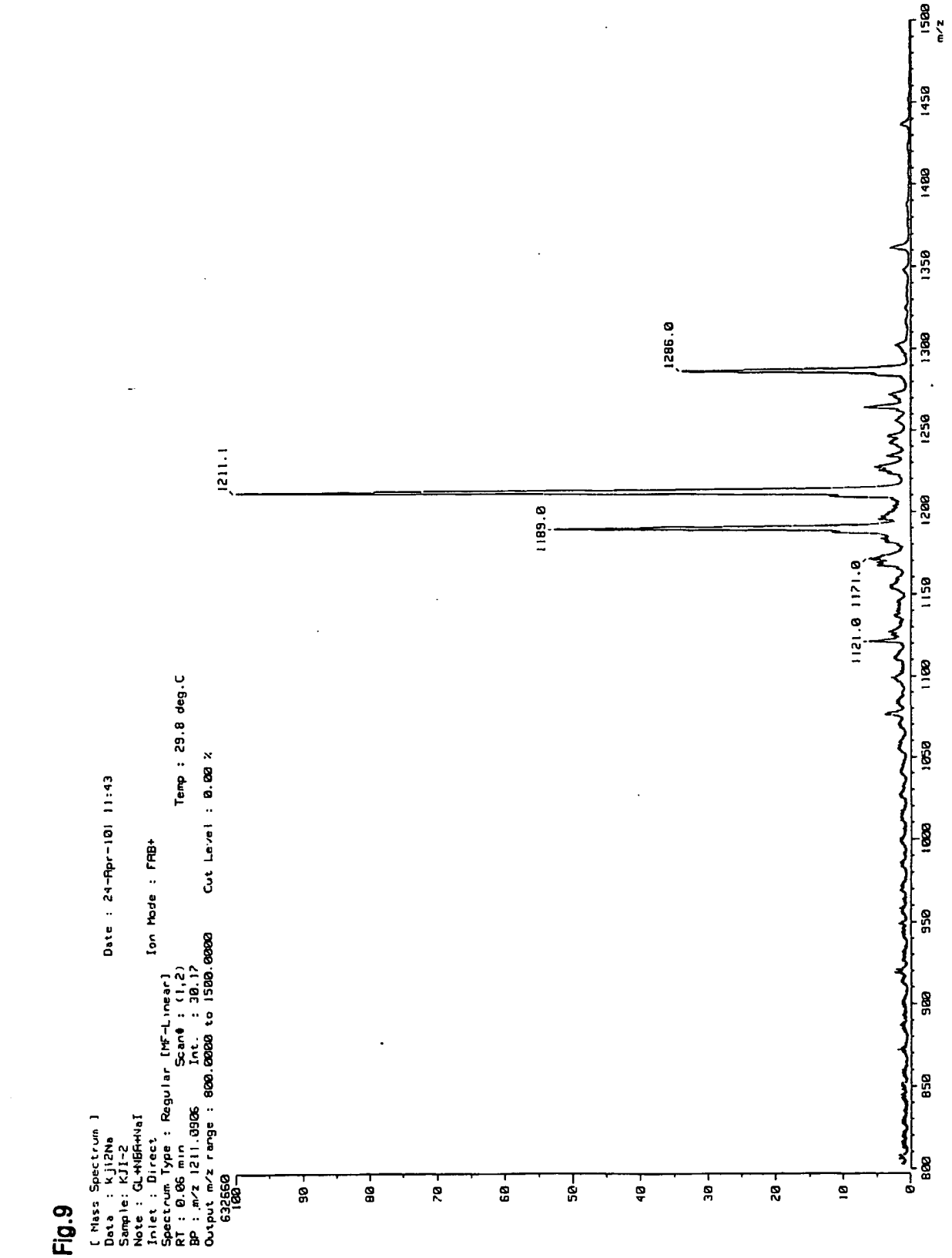
150
175
200
ppm

Fig.8

Fig.9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BP : m/z 1211.0906
Output m/z range : 800.0000 to 1500.0000
Date : 24-Apr-101 11:43
Ion Mode : FAB+
Scan# : (1,2)
Int. : 30.17
Temp : 29.8 deg.C
Cut Level : 0.00 %
632660
1211.1
1189.0
1171.0
1121.0
1286.0
m/z

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00880

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

KR, JP ; IPC as above

Electronic data base consulted during the internationl search (name of data base and, where practicable, search terms used)

CAPLUS(STN), MEDILINE(STN), USPATFULL, NPS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5807820 A (Novatis AG) 15 SEPTEMBER 1998 See column 1. line 53 - column 1. line 57. Claim 1	1 - 17
A	US 5284826 A (Sandoz Ltd.) 8 FEBRUARY 1994 See claims 1 - 6	1 - 17
A	James P. Jacobs et al., "Use of topical minoxidil therapy for androgenetic alopecia in women", International Journal of Dematology, 1993, Vol. 32, No. 10, pages 758 - 762 See the whole document	1 - 17
A	Maurer M. et al., "Hair growth modulation by topical immunophilin ligands : Induction of anagen, inhibition of massive catagen development and relative protection from chemotherapy-induced alopecia", 1997, Vol. 150, No. 4, pages 1433 - 1442 See the whole document	1 - 17
A	Yamamoto S. et al., "Hair growth stimulating effects of cyclosporin A and FK506, potent immunosuppressants", 1994, Vol. 7 suppl., pages S47 - S52 See the whole document	1 - 17

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 SEPTEMBER 2002 (19.09.2002)	19 SEPTEMBER 2002 (19.09.2002)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00880

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
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